Abstract of Master's Thesis, 2016

Study on Raman spectral alterations for early detection of apoptosis in human pancreatic cancer cell stimulated by caffeine and 5-fluorouracil

Graduate School of Science and Technology, Kwansei Gakuin University

Dept. of Bioscience, Sato Lab., Majed Esmael Jan

The purpose of this study is to analyze the origin of Raman spectral changes and to develop a method for early detection of apoptosis. Visual observation cannot detect the early stage of apoptosis. In contrast, it is necessary to use staining method for detection the early stage of cell death. Raman spectroscopy was applied in this study to improve the detection of apoptosis. Raman spectroscopy is capable to detect the very early reaction in living cells due to chemical and/or physical stimulations by analyzing their molecular changes. Raman scattering is based on the inelastic collision of photons to molecular electrons, as it is possible to obtain information of molecular composition without any preparation. Anticancer drugs inhibit the uncontrolled proliferation by suppressing the cell cycle and induce apoptosis to cancer cells. According to many researches, it has been found that caffeine accelerates a certain type of anticancer drug and inhibits the activities of some important protein kinases involved in DNA damage-induced cell cycle arrest and apoptosis. In this study, it has been demonstrated that confocal Raman microscopy (cRm) is suitable and reliable to observe molecular alteration in the living cells. Confocal Raman microscopy was used to observe biochemical changes induced by caffeine and 5-fluorouracil (5-FU) with different concentrations. The cells were exposed to the chemicals administrated into the cultivation dishes for 24 and 48 hours. Human pancreatic cancer cell was employed for a model sample, which was stimulated by caffeine and 5-FU. Two-dimensional gel electrophoresis (2DE) was applied to analyze the protein changes due to caffeine exposure. Tunel and Immunocytochemistry assays were applied for detecting the apoptosis and the morphological changes in BxPC-3. For the early detection of apoptosis, a single cell of BxPC-3 was exposed with 8 mM caffeine and observed by cRm at 0, 2, 4, and 6 hours. It was strongly suggested that cRm was powerful for early detection in molecular alteration induced by caffeine and 5-FU in living cells. Caffeine has made changes to the living cancer cell BxPC-3 in 2 hours as well as in different concentrations. It was succeeded also to detect the cell alterations with FTIR. Interestingly, there were no clear differences observed visually in the same sample, and what happens in the nucleus of the cell exposed to caffeine is not clear until now. Spots were observed in similar places but different concentration of proteins in the gels of 2DE between the cells treated with and without caffeine. An early apoptosis stage of BxPC-3 was observed by confocal Raman spectroscopy, even though there was no sign of apoptosis detected by optical observation microscope at the early hours after the caffeine exposure. The present results suggest spectral alterations for both DNA and protein. According to the cell monitoring, Raman spectroscopy detected a sign of apoptosis at the early hours of the treatment. On the other hand, it took 12 hours to observe it under the optical microscope. Thus, Raman spectroscopy is fast and reliable instrument for the early apoptosis detection.